GB00/01813

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

4

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Andrew Gersey

Dated 6 June 2000

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Patents Form 1/77

Patents Act 1
(Rule 16)

11 MAY 1999

The Patent Office

1[illegible]99 E446433-2 D00027
P01/7700 0.00-[illegible]91093.

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office
Cardiff Road
Newport
Gwent NP9 1RH

1. **Your reference** — 8.41.69960

2. **Patent application number** *(The Patent Office will fill in this part)* — 11 MAY 1999 — 9910934.0

3. **Full name, address and postcode of the or of each applicant** *(underline all surnames)*

Research Institute for Medicine
and Chemistry Inc.
49 Amherst Street
Cambridge
Massachusetts 02142
USA

Patents ADP number *(if you know it)* 685362001 Rdes

If the applicant is a corporate body, give country/state of incorporation — USA

4. **Title of the invention** — Chemical Compounds

5. **Name of your agent** *(if you have one)* — Frank B. Dehn & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

179 Queen Victoria Street
London
EC4V 4EL

Patents ADP number *(if you know it)* — 166001

6. **If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and** *(if you know it)* **the or each application number**

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. **If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application**

Number of earlier application	Date of filing *(day / month / year)*

8. **Is a statement of inventorship and of right to grant of a patent required in support of this request?** *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Patents Form 1/77

9. **Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document**

Continuation sheets of this form	0
Description	26
Claim(s)	0
Abstract	0
Drawing(s)	0

10. **If you are also filing any of the following, state how many against each item.**

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	-
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(please specify)*	-

11. **I/We request the grant of a patent on the basis of this application.**

Signature **Date** 11 May 1999

Frank B. Dehn & Co.

12. **Name and daytime telephone number of person to contact in the United Kingdom**

J C Marsden
0171 206 0600

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*
b) *Write your answers in capital letters using black ink or you may type them.*
c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s) of the form. Any continuation sheet should be attached to this form.*
d) *If you have answered 'Yes', Patents Form 7/77 will need to be filed.*
e) *Once you have filled in the form you must remember to sign and date it.*
f) *For details of the fee and ways to pay please contact the Patent Office.*

69960.604

Chemical Compounds

This invention relates to novel sterol derivatives, more particularly to ring A aromatic sterol derivatives in which the 17-position side chain terminates in an amine or amide group and which exhibit cell modulating activity.

It is well known that 9,10-seco sterol derivatives such as vitamin D_3 play a vital role in the metabolism of calcium by promoting intestinal absorption of calcium and phosphorus, maintaining adequate serum levels of calcium and phosphorus, and stimulating mobilisation of calcium from the bone fluid compartment in the presence of parathyroid hormone. Following the discovery that D vitamins are hydroxylated *in vivo*, at the 25-position in the liver and at the 1α-position in the kidneys, and that the resulting 1α,25-dihydroxy metabolite is the biologically active material, extensive studies have been carried out on vitamin D analogues hydroxylated at, for example, the 1α- and 24R- or 25-positions.

The natural metabolite 1α,25-dihydroxy vitamin D_3 has additionally been found to have effects on cellular metabolism, these cell modulating effects including stimulation of cell maturation and differentiation, immunosuppressive effects and immunopotentiating effects (e.g. by stimulating the production of bactericidal oxygen metabolites and the chemotactic response of leukocytes). However, the potent effects of compounds such as 1α,25-dihydroxy vitamin D_3 on calcium metabolism will normally preclude their use in this area, since doses sufficient to elicit a desired cell modulating effect will tend to lead to unacceptable hypercalcaemia.

This has led to attempts to synthesize new vitamin D analogues which have reduced effects on calcium metabolism but which still exhibit the desired effects on cellular metabolism. Representative examples of such

analogues, together with summaries of earlier attempts to solve this problem, are given in WO-A-9309093, WO-A-9426707, WO-A-9525718 and WO-A-9516672, the contents of which are incorporated herein by reference.

It is currently believed that such vitamin D analogues act as general regulators of cell growth and differentiation through receptor-mediated (especially nuclear receptor-mediated) processes involving modulation of vitamin D responsive genes (M.R. Waters, Endoc. Rev. **13**, pp. 719-764 [1992]). It has also hitherto been assumed that the seco steroid 5,7,10(19)-triene system or a similar 19-nor seco steroid 5,7-diene system is a prerequisite for any form of cell modulating activity. Thus, whilst workers investigating vitamin D analogues have modified the A-ring and 17-position side chain and in certain cases have made more drastic modifications to the overall molecular skeleton such as modification or even elimination of the C- and/or D-rings, they have attempted to retain the triene or conjugated diene system (Gui-Dong Zhu *et al.*, Bioorganic & Med. Chem. Lett. **6**, pp. 1703-1708 [1996]; K. Sabbe *et al.*, Bioorganic & Med. Chem. Lett. **6**, pp. 1697-1702 [1996]).

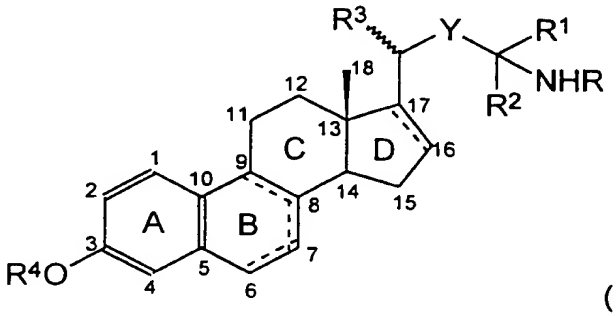
Workers have recently reported the observation of non-genomic rapid responses to vitamin D analogues which they attribute to interaction with a putative cell membrane-located vitamin D receptor (A.W. Norman *et al.*, J. Steroid Biochem. and Mol. Biol. **56**, pp. 13-22 [1996]). It has also been reported that such non-genomic rapid effects may be elicited by 1α,3β,25-trihydroxycholesta-5,7-diene, i.e. the pro-vitamin form of 1α,25-dihydroxy vitamin D_3, which is not a seco steroid; this has been attributed to the ability of the pro-vitamin to mimic the 6,7-s-cis conformation of the normal vitamin D triene (Norman, *op. cit.*). However, the pro-vitamin has been reported to have little ability to elicit the genomic effect believed to underlie

modulation of cell growth and differentiation (Norman, *op. cit.*) and has also been reported not to exhibit the typical effects of vitamin D on skin (R. Gniadecki *et al.*, British J. Dermatol. **132**, pp. 841-852 [1995]).

The present invention is based on the surprising finding that a range of simple sterol derivatives which have an intact tetracyclic nucleus and thus lack both the seco steroid triene system of vitamin D analogues and the ability to mimic a conjugated conformational isomer thereof, exhibit potent effects on the modulation of cell growth and differentiation, for example as demonstrated by their ability to inhibit growth of cancer cells *in vitro* and *in vivo*, and their ability to promote the healing of ear punches *in vivo*. The compounds possess an advantageous therapeutic ratio by virtue of their low levels of calcaemic activity, for example as determined by their effects on serum calcium and phosphorus levels in rats.

The compounds of the invention comprise 3-sterols (and O-protected derivatives thereof) having an aromatic A ring and an amine- or amide-terminated 17-position side chain. The compounds may also contain an aromatic B-ring or a double bond at the 7(8)position and/or a double bond at the 16(17)-position.

Thus according to one embodiment of the invention there are provided compounds of formula (I)



(I)

in which:

R represents a hydrogen atom, an aliphatic, cycloaliphatic, araliphatic or aryl organic group, or an acyl group comprising such an organic group linked to the nitrogen atom by way of a carbonyl group;

R^1 and R^2, which may be the same or different, each represents a lower alkyl or cycloalkyl group, or together with the carbon atom to which they are attached form a lower cycloalkyl group;

R^3 represents a methyl group having α- or β-configuration;

R^4 represents a hydrogn atom or an O-protecting group;

Y represents a lower alkylene, alkenylene or alkynylene group optionally substituted by a hydroxyl, etherified hydroxyl or esterified hydroxyl group; and

the dotted lines signify that double bonds may be present at the 16(17)-position and/or either at the 6(7)- and 8(9)-positions or at the 7(8) position.

Where R represents an aliphatic group this may, for example, be a lower alkyl group, for example a C_{1-6} alkyl group such as a methyl, ethyl, propyl or butyl group. Where R is cycloaliphatic it may, for example, be a lower (e.g. C_{3-8}) cycloalkyl group such as cyclopropyl, cyclopentyl or cyclohexyl. Araliphatic groups R may, for example, include C_{6-12} carbocyclic aryl C_{1-4} alkyl groups such as benzyl or phenethyl; aryl groups may, for example, include C_{6-12} carbocyclic aryl groups such as phenyl or naphthyl. Where R represents an acyl group this may, for example, be a lower (e.g. C_{1-6}) alkanoyl group such as formyl, acetyl or propionyl; a C_{6-12} carbocyclic aryl C_{2-5} alkanoyl group such as phenylacetyl; or a C_{7-13} carbocyclic aroyl group such as benzoyl. The group R may optionally carry one or more substituents, for example selected from halo (e.g. chloro or bromo), lower (e.g. C_{1-4}) alkyl such as methyl, lower alkoxy (e.g. methoxy), lower alkanoyl (e.g.

acetyl), lower alkylamino (e.g. methylamino), di(lower alkyl)amino (e.g. dimethylamino), nitro, carbamoyl and lower alkanoylamino (e.g. acetamido).

Aliphatic groups represented by R^1 and R^2 may, for example, include lower (e.g. C_{1-6}) alkyl groups such as methyl, ethyl, propyl and butyl groups. Cycloaliphatic groups may, for example, include lower (e.g. C_{3-8}) cycloalkyl groups such as cyclopropyl, cyclopentyl and cyclohexyl groups.

Where R^3 in formula (I) is a methyl group in the α-configuration, the compounds have the 20R configuration characteristic of natural sterols such as cholesterol; where R^3 is in the β-configuration the compounds have the 20S configuration of the corresponding epi-derivatives. It will be appreciated that the invention also embraces mixtures of the two isomers.

Where R^4 represents an O-protecting group this may, for example, comprise any suitable cleavable O-protecting group such as is commonly known in the art. Representative groups include (i) etherifying groups such as silyl groups (e.g. tri(lower alkyl)silyl groups such as trimethylsilyl, triethylsilyl, triisopropylsilyl or t-butyldimethylsilyl; tri(aryl)silyl groups such as triphenylsilyl; and mixed alkyl-arylsilyl groups), lower (e.g. C_{1-6}) alkyl groups optionally interrupted by an oxygen atom (e.g. such as methyl, methoxymethyl or methoxyethoxymethyl) and cyclic ether groups (e.g. such as tetrahydropyranyl), and (ii) esterifying groups such as lower (e.g. C_{1-6}) alkanoyl (e.g. such as acetyl, propionyl, isobutyryl or pivaloyl), aroyl (e.g. containing 7-15 carbon atoms, such as benzoyl or 4-phenylazobenzoyl), lower (e.g. C_{1-6}) alkane sulphonyl (e.g. such as methane sulphonyl or halogenated methane sulphonyl) and arene sulphonyl (e.g. such as p-toluene sulphonyl).

Such O-protected derivatives of compounds of formula (I) are useful in the preparation of active

compounds (I) in which R^1 represents a hydroxy group and may also, where the O-protecting group is metabolically labile *in vivo*, be useful directly in therapy.

Lower alkylene, alkenylene or alkynylene groups represented by Y may, for example, contain up to 7 carbon atoms and up to 3 multiple bonds. Y may advantageously be a straight chained group, e.g. containing 3-6 carbon atoms, for example as in trimethylene, tetramethylene, pentamethylene, hexamethylene, buta-1,3-dienylene, propynylene, but-1-ynylene or but-2-ynylene.

Where Y is substituted by a hydroxyl, etherified hydroxyl or esterified hydroxyl group, this substituent may advantageously be positioned α-, β- or γ- to the group $-C(R^1)(R^2).NHR$ or α- to any triple bond present in the group Y. Etherified hydroxyl groups which may be present inclkude lower (e.g. C_{1-6}) alkyl groups optionally interrupted by one or more oxygen atoms (e.g. methyl, methoxymethyl or methoxyethoxymethyl), and cyclic groups such as tetrahydropyranyl. Esterified hydroxyl groups which may be present include lower (e.g. C_{1-6}) alkanoyl such as acetyl, propionyl, isobutyryl or pivaloyl; lower alkenoyl (e.g. allylcarbonyl); aroyl (e.g. p-nitrobenzoyl); lower alkoxycarbonyl (e.g. t-butoxycarbonyl); lower haloalkoxycarbonyl (e.g. 2,2,2-trichloroethoxycarbonyl or 1,1,1-trichloro-2-methyl-2-propoxycarbonyl); aralkyloxycarbonyl (e.g. benzyloxycarbonyl or p-nitrobenzyloxycarbonyl); and lower alkenyloxycarbonyl (e.g. allyloxycarbonyl). It will be appreciated that it may be advantageous to select etherifying or esterifying groups which are metabolically labile *in vivo*.

The cell modulating activity of compounds according to the invention, including O-protected derivatives in which the O-protecting group is metabolically labile, combined with their substantial lack of calcaemic effect, render them of interest both alone and as

adjuncts in the management of diseases associated with abnormal cell proliferation, such as neoplastic disease, particularly myelogenous leukemias as well as neoplastic disease of the brain, breast, stomach, gastrointestinal tract, prostate, pancreas, uro-genital tract (male and female) and pulmonary neoplasia. Their ability to promote closure of mouse ear punches suggests their use, either alone or as adjuncts, as agents to promote wound healing. Their cell modulating activity suggests that these compounds may, like vitamin D analogues, have additional utilities either alone or as adjuncts in the chemotherapy of infection and in other therapeutic modalities in which mononuclear phagocytes are involved, for example in treatment of bone disease (e.g. osteoporosis, osteopenia and osteodystrophy as in rickets or renal osteodystrophy), autoimmune disease, host-graft reaction, transplant rejection, inflammatory diseases (including modulation of immunoinflammatory reactions), neoplasias and hyperplasias, myopathy, enteropathy and spondylitic heart disease, their potential utility in treatment of neoplasias and hyperplasias being evidenced by their ability to inhibit human cancer xenografts in severe combined immunodeficiency mice. Additionally, they may be useful in suppression of parathyroid hormone (e.g. as in serum calcium homeostasis), in treatment of dermatological diseases (for example including acne, alopecia, eczema, pruritus, psoriasis and skin aging, including photoaging), hypertension, rheumatoid arthritis, psoriatic arthritis, secondary hyperparathyroidism, asthma, cognitive impairment and senile dementia (including Alzheimer's disease), in fertility control in both human and animal subjects, and in management of disorders involving blood clotting (e.g. by dissolution of existing clots and/or by prevention of clotting). The invention embraces use of these compounds in the therapy or prophylaxis of such conditions and in the

manufacture of medicaments for use in such treatment or prophylaxis.

Active compounds according to the invention may be formulated for administration by any convenient route, e.g. orally (including sublingually), parenterally, rectally or by inhalation; pharmaceutical compositions so formulated comprise a feature of the invention.

Orally administrable compositions may, if desired, contain one or more physiologically compatible carriers and/or excipients and may be solid or liquid. The compositions may take any convenient form including, for example, tablets, coated tablets, capsules, lozenges, aqueous or oily suspensions, solutions, emulsions, syrups, elixirs and dry products suitable for reconstitution with water or another suitable liquid vehicle before use. The compositions may advantageously be prepared in dosage unit form. Tablets and capsules according to the invention may, if desired, contain conventional ingredients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth or polyvinyl-pyrollidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as sodium lauryl sulphate. Tablets may be coated according to methods well known in the art.

Liquid compositions may contain conventional additives such as suspending agents, for example sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxymethylcellulose, carboxymethylcellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate or acacia; non-aqueous vehicles, which may include edible oils, for example vegetable oils such as arachis oil, almond oil, fractionated coconut oil, fish-liver oils, oily esters such as polysorbate 80,

propylene glycol, or ethyl alcohol; and preservatives, for example methyl or propyl p-hydroxybenzoates or sorbic acid. Liquid compositions may conveniently be encapsulated in, for example, gelatin to give a product in dosage unit form.

Compositions for parenteral administration may be formulated using an injectable liquid carrier such as sterile pyrogen-free water, sterile peroxide-free ethyl oleate, dehydrated alcohol or propylene glycol or a dehydrated alcohol/propylene glycol mixture, and may be injected intravenously, intraperitoneally or intramuscularly.

Compositions for rectal administration may be formulated using a conventional suppository base such as cocoa butter or another glyceride.

Compositions for administration by inhalation are conveniently formulated for self-propelled delivery, e.g. in metered dose form, for example as a suspension in a propellant such as a halogenated hydrocarbon filled into an aerosol container provided with a metering dispense valve.

It may be advantageous to incorporate an antioxidant, for example ascorbic acid, butylated hydroxyanisole or hydroquinone in the compositions of the invention to enhance their storage life.

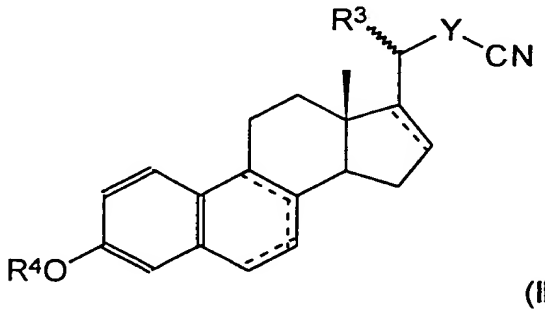
Where any of the above compositions are prepared in dosage unit form these may for example contain 100 μg - 100 mg of active compound according to the invention per unit dosage form, such dosage units may for example be administered 1-4 times per day. The compositions may if desired incorporate one or more further active ingredients.

A suitable daily dose of an active compound according to the invention may for example be in the range 100 μg - 400 mg, per day, depending on factors such as the severity of the condition being treated and the age, weight and condition of the subject.

Compounds according to the invention may be prepared by any convenient method, for example by reaction of a compound containing a precursor for the desired side chain in one or more stages and with one or more reactants serving to form the desired 17-position side chain, followed if necessary and/or desired by removal of any O-protecting group.

Appropriate techniques for formation of a desired side chain include those described in the aforementioned WO-A-9516672.

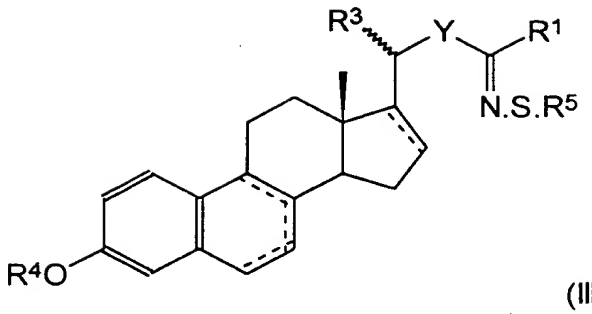
Thus, for example, in order to prepare a compound (I) in which R^1 and R^2 are the same, a compound of general formula (II)



(II)

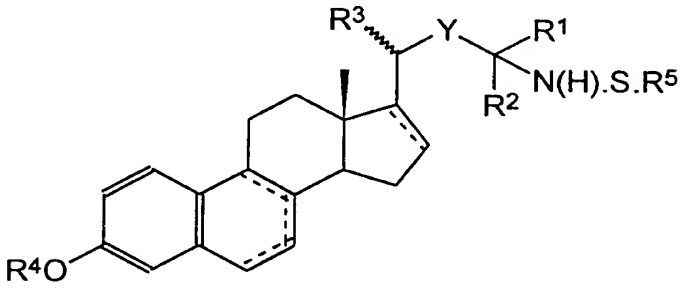
(where R^3, R^4 and Y are as hereinbefore defined) may be reacted with an organo-cerium reagent, e.g. prepared <u>in situ</u> from cerous chloride and an appropriate organometallic compound, e.g. an alkyl/cycloalkyl lithium compound of formula R^1Li (where R^1 is as hereinbefore defined), for example as described by Ciganek (J. Org. Chem. **57**, pp. 4521-4527 [1992]).

Compounds of formula (I) in which R^1 and R^2 are different may, for example, be prepared by reacting a thio-oxime of formula (III)



(III)

(where R^1, R^3, R^4 and Y are as hereinbefore defined and R^5 is an aromatic group, e.g. a carbocyclic aryl group such as phenyl) with an appropriate organometallic compound, for example an alkyl/cycloalkyl lithium compound of formula R^2Li (where R^2 is as hereinbefore defined), and reducing the thus-obtained compound of formula (IV)



(IV)

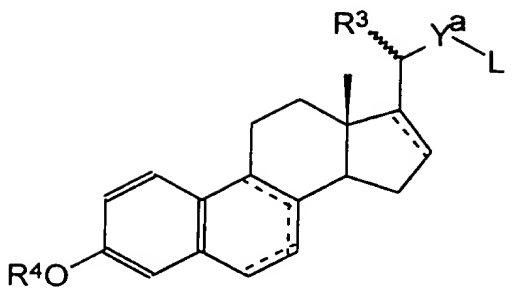
(where R^1, R^2, R^3, R^4, R^5 and Y are as hereinbefore defined), e.g. using a metal hydride reducing agent such as sodium borohyride or an inorganic or organic sulphur compound such as hydrogen sulphide, sodium sulphide or a thiol (e.g. a lower alkyl mercaptan such as methanethiol) to remove the $R^5.S$ group and yield a corresponding compound of formula (I) in which R is a hydrogen atom (see J. Org. Chem. **42**, pp. 398-399

[1977]).

Compounds of formula (I) in which R represents a lower alkanoyl, aralkanoyl or aroyl group may be prepared by acylation of a corresponding compound (I) in which R is hydrogen, for example by reaction with an appropriate acyl halide or acid anhydride, preferably in the presence of water or a lower alcohol, as may typically be incorporated to suppress acylation of groups other than the amino group, or with an appropriate acid in the presence of a coupling agent such as N,N'-carbonyl-diimidazole or dicyclohexylcarbodiimide. It will be appreciated that, if the acylation is carried out in the absence of components such as water or lower alcohols which suppress the acylation of hydroxyl groups, any hydroxyl groups present in the molecule, either at the 3-position or as a substituent of the Y group, should desirably be in O-protected form during such an acylation reaction.

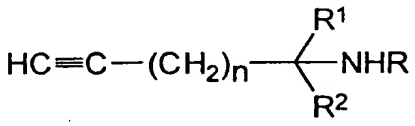
Compounds of formula (I) in which R represents a lower alkyl group may, for example, be prepared by reducing a corresponding compound (I) in which R is a lower alkanoyl group, e.g. using a metal hydride reducing agent such as lithium aluminium hydride. Alternatively a compound (I) in which R represents a hydrogen atom may be subjected to direct alkylation, e.g. by reaction with an alkyl halide, or to reductive amination, e.g. by reaction with an appropriate aldehyde and a reducing agent such as sodium cyanoborohydride.

Compounds of formula (I) in which Y is an alkynylene group may, for example, be prepared by reaction of a compound of formula (V)



(V)

(where R^3 and R^4 are as hereinbefore defined; Y^a is an alkylene group, e.g. containing 1-4 carbon atoms; and L represents a leaving group, for example a sulphonate ester group, e.g. lower alkyl sulphonyloxy such as mesyloxy, lower fluoroalkyl sulphonyloxy such as trifluoromethanesulphonyloxy or aryl sulphonyloxy such as tosyloxy, or a halogen atom such as chlorine, bromine or iodine), with a metallated derivative (e.g. the lithio derivative) of an alkyne of formula (VI)



(VI)

(where R, R^1 and R^2 are as hereinbefore defined and n is 0 or an integer, e.g. in the range 1-3).

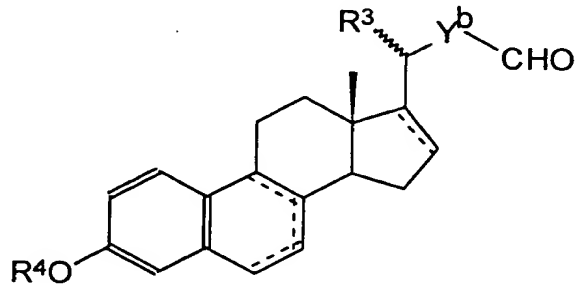
The thus obtained compound (I) in which Y is the group

$$-Y^a-C\equiv C-(CH_2)_n-$$

(wherein Y^a and n are as hereinbefore defined) may if desired be hydrogenated to convert the triple bond either to a double bond (e.g. using Lindlar catalyst) or to a single bond (e.g. using a noble metal catalyst such

as platinum, palladium or homogeneous rhodium or ruthenium).

Compounds of formula (I) in which Y is an alkynylene group carrying a hydroxyl group α to the triple bond may, for example, be prepared by reaction of a compound of formula (VII)



(VII)

(where R^3 and R^4 are as hereinbefore defined and Y^b is a valence bond or an alkylene group, e.g. containing 1-4 carbon atoms) with a metallated derivative of an alkyne of formula (VI), so as to form a compound (I) in which Y is a group

$$-Y^b-CH(OH)-C\equiv C-(CH_2)_n-$$

(wherein Y^b and n are as hereinbefore defined).

Compounds of the formula (VI) may be prepared by subjecting a compound of formula (VIII)

$$CH_3(CH_2)_nC\equiv C-C(R^1)(R^2)OH \qquad (VIII)$$

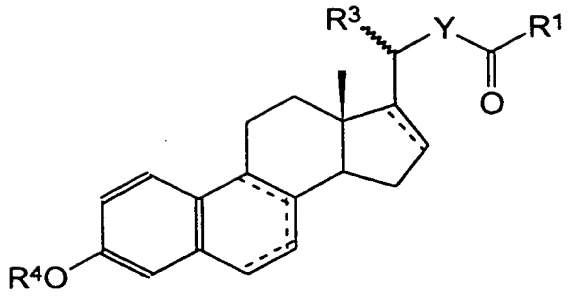
(where n, R^1 and R^2 are as hereinbefore defined) to a Ritter reaction with a compound of formula R^aCN (where R^a represents a hydrogen atom or an appropriate organic group) in the presence of a strong acid, e.g. a mineral acid such as sulphuric acid, thereby leading to formation of a compound (I) in which R represents a

group $R^a.CO$-. This group may be removed by hydrolysis to yield a compound (I) in which R represents a hydrogen atom or may be reduced, e.g. as hereinbefore described, to yield a compound (I) in which R represents a group $R^a.CH_2$-. Alternatively the hydroxyl group of the tertiary carbinol may be displaced by an azido group, e.g. by reaction with hydrazoic acid in the presence of a strong acid, and the azido group reduced to yield a compound (I) in which R represents hydrogen. The internal alkyne may then be isomerized to the terminal position by treatment with the potassium salt of 1,3-propanediamine in 1,3-propanediamine as solvent ("acetylene zipper").

Compounds of formula (II) may, for example, themselves be prepared by reaction of a compound of formula (V) as defined above with, as appropriate, (i) a source of cyanide ion (e.g. an alkali metal cyanide such as sodium or potassium cyanide), (ii) a metallated acetonitrile derivative (e.g. the lithio derivative), or (iii) acrylonitrile, preferably where L is an iodine atom (e.g. by ultrasound-induced chromium-mediated conjugate addition as described by Mourino et al. in J. Org. Chem. 58, pp. 118-123 [1993]).

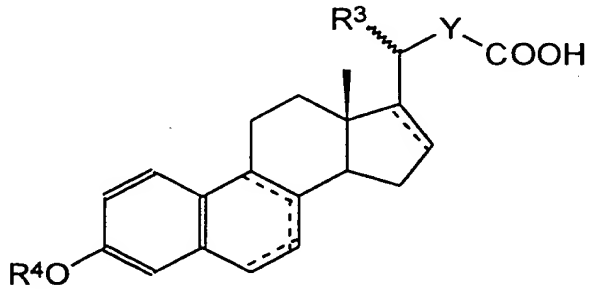
Compounds (II) in which the 17-position side chain terminates in the group -CH:CH.CN may, for example, be prepared from an aldehyde of formula (VI) as defined above by means of a Wittig reaction with an ylid of formula $(R^9)_3P$:CH.CN (where each R^9 represents an organic group, e.g. a carbocyclic aryl group such as phenyl) or with a corresponding phosphonate or silyl equivalent.

Compounds of formula (III) may, for example, themselves be prepared by reacting a ketone of formula (IX)



(IX)

(where R^1, R^3, R^4 and Y are as hereinbefore defined) with an S-substituted thiolamine of formula $R^5.S.NH$ (where R^5 is as hereinbefore defined). Such compounds of formula (IX) may be prepared from, for example, an acid of formula (X)



(X)

(where R^3, R^4 and Y are as hereinbefore defined), e.g. by formation of a corresponding acid halide such as the chloride and reaction with an organometallic compound R^1MX (where R^1 is as hereinbefore defined; M represents a divalent metal such as copper, zinc or cadmium; and X represents e.g. a halogen atom). Alternatively one may prepare compounds (IX) by reacting a compound of formula (V) above with e.g. (i) an α-metallated derivative such as a lithio derivative of a ketone of formula $CH_3.CO.R^1$ (where R^1 is as hereinbefore defined) or with a

corresponding enol, or (ii), preferably where L is an iodine atom, a vinyl ketone of formula $CH_2:CH.CO.R^1$ (where R^1 is as hereinbefore defined), e.g. by ultrasound-induced chromium-mediated conjugate addition as described by Mourino et al. (op. cit.).

Compounds (X) and esters thereof may also be used to prepare compounds of formula (II) by reaction with ammonia or a metallated derivative thereof, e.g. an alkali metal amide such as lithium amide, to form a corresponding carboxamide which may be converted to a nitrile (II) by mild dehydration, e.g. using tosyl chloride, phosphorus oxychloride in the presence of a base such as pyridine, or trifluoracetic anhydride in the presence of an excess of a base such as pyridine.

Compounds (II) in which Y is α-substituted by a hydroxyl group are conveniently obtained by cyanohydrin formation, for example by reaction of a compound (VII) with hydrogen cyanide. Compounds (II) in which Y is β-substituted by a hydroxyl group may be prepared directly by reaction of a compound (VII) with a metallated (e.g. lithated) derivative of acetonitrile; they may also be prepared indirectly by reaction with a metallated derivative of an ester of acetic acid, followed by conversion of the ester grooup to a carboxamide group and then to a nitrile group, e.g. as described above.

In general compounds (I) and starting materials therefor in which Y is substituted by a hydroxyl group may be converted to corresponding ether and ester derivatives by standard methods such as are well known in the art. Thus, for example, etherification may be effected by reaction with an appropriate organic halide (e.g. an alkyl iodide) in the presence of an appropriate base (e.g. an alkali metal alkoxide such as potassium t-butoxide), advantageously in the presence of a crown ether such as 18-crown-6. Esterification may be effected by reaction with appropriate acylating agents, such as acyl halides, acid anhydrides and the like.

Compounds of formula (V) may be prepared from estrone, equilenin or equilin as appropriate by, for example, Wittig reaction with an ethylidene phosphorane to convert the 17-one to the corresponding Z-17(20) ethylidene compound, following the procedure described by Krubiner and Oliveto, J. Org. Chem. **31**, pp. 24-26 [1965]. Alternatively, the corresponding E-isomer may be obtained following the procedure of Midland and Kwon, Tetrahedron Lett. **23(20)**, pp. 2077-2080 [1982]. The thus-obtained alkenes may be subjected to conventional stereospecific hydroboration reactions followed by oxidative work-up with alkaline hydrogen peroxide solution (Krubiner, *op. cit.*) to afford the corresponding 20-ols, which may be oxidised to 20-ones with chromium trioxide (Krubiner, *op. cit.*). Wittig reaction with methoxymethylenetriphenylphosphorane, hydrolysis of the enol ether with aqueous acid (to give a non-stereospecific aldehyde of formula (VII) in which Y_b represents a valence bond), reduction with sodium borohydride and reaction of the resulting alcohol with tosyl chloride affords compounds of formula (V) wherein R^3 is methyl, Y^a is methylene and L is tosyloxy.

Compounds of formula (V) having a double bond at the 16(17)-position may, for example, be prepared stereospecifically by subjecting the appropriate E- or Z-17(20) ethylidene compound prepared as described above to a stereospecific ene reaction. For example, such ene reactions include treatment with formaldehyde, boron trifluoride and optionally acetic anhydride (Batcho *et al.*, Helv. Chim. Acta **64**, pp. 1682-1687 [1981]) to form compounds of formula (V) in which R^3 is methyl, Y^a is methylene and L is hydroxy or acetoxy. The acetyl group may be removed by hydrolysis and the hydroxyl group may be tosylated to generate a compound (V) in which L is a suitable leaving group. In an alternative ene reaction, treatment with ethyl propiolate / diethyl aluminium chloride (Dauben and Brookhart, J. Am. Chem. Soc. **103**,

pp. 237-238 [1980]) affords ethyl esters of Δ16,17 acids of general formula (X) in which R^3 is methyl and Y is ethylene, from which the corresponding free acid may be obtained by hydrolysis. The Δ16,17 compounds described above may be stereospecifically hydrogenated.

Compounds of formula (V) in which Y^a is e.g. ethylene or trimethylene may, for example, be obtained by reaction of a compound (V) in which Y^a is methylene either (i) with a reagent serving to introduce a one-carbon fragment (e.g. a metal cyanide) and conversion of the group so introduced to a group $-CH_2L$, e.g. by hydrolysing a cyano group to yield a carboxy group or by reducing such a cyano group (e.g. with a metal hydride reducing agent such as diisobutyl aluminium hydride) to yield a carboxaldehyde group, and reducing the carboxy or carboxaldehyde group (e.g. using sodium borohydride or lithium aluminium hydride) to yield a hydroxymethyl group which may in turn be subjected to tosylation and, if desired, nucleophilic displacement as hereinbefore described to effect conversion to a halomethyl group; or (ii) with a metallated derivative of an ester or thioester of acetic acid, with a derivative containing another carbanionic equivalent of acetic acid (e.g. a metallated derivative of acetonitrile), or with a metallated malonate ester (in which last instance the reaction product is partially hydrolysed to yield a monoester which may be decarboxylated by heating to yield a carboxylate ester), reducing the resulting ester or thioester product to an alcohol (e.g. using lithium aluminium hydride), and converting the resulting hydroxyl group to a leaving group, such as a tosylate group or a halogen atom, e.g. as hereinbefore described.

It will be appreciated that the above procedures (i) and/or (ii) may be repeated as needed to yield compounds (V) in which Y^a is a C_3 - C_7 alkylene group.

In general, O-protecting groups may, for example, be removed by conventional methods such as are well

documented in the literature. Thus esterifying acyl groups may be removed by basic hydrolysis, e.g. using an alkali metal alkoxide in an alkanol. Etherifying groups such as silyl groups may be removed by acid hydrolysis or treatment with a fluoride salt, e.g. a tetraalkyl ammonium fluoride. The use of such acid-labile but base-stable protecting groups may be of particular advantage during homologation steps to build up a desired side chain, in view of the strongly basic conditions normally employed for such reactions.

The following non-limitative examples serve to illustrate the invention. All temperatures are in °C.

Preparation 1

a) 3-Triisopropylsilyloxy-19-nor-chol-1,3,5(10),16-tetraen-24-ol [Formula (V) - R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y^a = $(CH_2)_3$, L = OH, Δ16 double bond]

A solution of 3-triisopropylsilyloxy-19-nor-chol-1,3,5(10),16,22-pentaene-24-carboxylic acid methyl ester [Formula (V) - R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y^a = -CH=CH-, L = $CO.OCH_3$, Δ16 double bond] (177 mg - prepared by silylation of the corresponding 3-hydroxy compound) in ether (6.5 ml) was added dropwise to a solution of lithium aluminium hydride in ether (3 ml of a 1M solution). The mixture was stirred for 3 hours and worked up to afford the title compound as an approximately 85/15 mixture with the corresponding Δ22 compound.

b) 3-Triisopropylsilyloxy-19-nor-chol-1,3,5(10),16-tetraen-24-ol, 24-tosylate [Formula (V) - R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y^a = $(CH_2)_3$, L = $O.SO_2.C_6H_4.CH_3$, Δ16 double bond

A solution containing the mixture of alcohols from (a) above (223 mg), tosyl chloride (216 mg) and pyridine (476 μl) in methylene chloride (4 ml) was stirred at room temperature for 4 hours, treated with aqueous sodium bicarbonate solution, stirred overnight, and worked up to afford a mixture of the Δ22 alcohol and the title compound (190 mg): NMR ($CDCl_3$) δ 0.85 (s, 18-H's), 2.65 (s, tosyl-Me), 3.9 (t, 24-H's), 5.1 (bs, 16-H), 6.5 and 6.95 (m, 1-, 2- and 4-H's), 7.65 and 7.62 (ABq, tosyl-H's).

c) 3-Triisopropylsilyloxy-19-nor-chol-1,3,5(10),16-tetraene-24-bromide [Formula (V) - R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y^a = $(CH_2)_3$, L = Br, Δ16 double bond

The 24-tosylate from (b) above (190 mg) in 1,2-dichloroethane (5 ml) and acetonitrile (5 ml) containing lithium bromide (300 mg) was heated under reflux for 3 hours. The reaction mixture was then cooled, diluted with ethyl acetate, washed with water then brine, and dried over sodium sulphate. Evaporation of the solvent gave the title product (156 mg): NMR ($CDCl_3$) δ 0.9 (s, 18-H's), 3.5 (t, 24-H's), 5.2 (bs, 16-H), 6.5 and 6.95 (m, 1-, 2- and 4-H's).

Preparation 2

a) 3-Triisopropylsilyloxy-19-nor-chol-1,3,5(10)-triene-24-carboxylic acid methyl ester [Formula (V) - R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y^a = $(CH_2)_2$, L = $CO.OCH_3$]

A solution of the Δ16, Δ22-pentaenic acid methyl ester used as starting material in Preparation 1(a) (200 mg) in ethyl acetate (10 ml) was treated with palladium/charcoal (400 mg, 10%) and stirred overnight under an atmosphere of hydrogen. Filtration through Celite and removal of the solvent under reduced pressure afforded the title compound (177 mg): NMR ($CDCl_3$) δ 0.96 (s, 18-H's), 3.7 (s, ester CH_3), 6.5 and 6.95 (m, 1-, 2- and 4-H's) (peaks at δ 5.2 and 5.6-5.9 were absent).

b) 3-Triisopropylsilyloxy-19-nor-chol-1,3,5(10)-triene-24-ol [Formula (V) - R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y^a = $(CH_2)_3$, L = OH]

The ester from (a) above (177 mg) was treated with lithium aluminium hydride (3 ml of a 1M solution in ether) for 3 hours at room temperature. The resulting

product was worked up to give the title compound (158 mg): NMR ($CDCl_3$) δ 3.9 (t, 24-H's), 6.5 and 6.95 (m, 1-, 2- and 4-H's) (peak at δ 3.8 was absent).

c) 3-Triisopropylsilyloxy-19-nor-chol-1,3,5(10), triene-24-bromide [Formula (V) - R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y^a = $(CH_2)_3$, L = Br]

Treatment of the alcohol from (b) above (158 mg) with tosyl chloride as in Preparation 1(b), followed by treatment of the resulting toluene sulphonate (176 mg) with lithium bromide as in Preparation 1(c) afforded the title compound (131 mg): NMR ($CDCl_3$) δ 0.96 (s, 18-H's), 3.4 (t, 24-H's), 6.5 and 6.95 (m, 1-, 2- and 4-H's).

Example 1

a) 3-Triisopropylsilyloxy-23,23a-bis-homo-19-nor-chol-1,3,5(10)16-tetraene-24-nitrile [Formula (II) - R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y = $(CH_2)_4$, Δ16 double bond]

A solution of acetonitrile (0.16 ml) in tetrahydrofuran (1.5 ml) was added dropwise at -78° to a solution of butyl lithium in hexane (3 mM in 1.9 ml) and the reaction mixture was stirred for 50 minutes. The bromide from Preparation 1(c) (150 mg) in tetrahydrofuran (3 ml + 1 ml wash) was added and the mixture was stirred for a further hour then warmed to -30° for an hour. TLC indicated the absence of starting material, so the mixture was cooled to -78° and treated with ammonium chloride. The product was extracted into ether and worked up to afford the title compound (85 mg): IR ν_{max} 2250, 1620 cm^{-1}; NMR ($CDCl_3$) δ 0.96 (s, 18-H's), 5.2 (bs, 16-H), 6.5 and 6.95 (m, 1-, 2- and 4-H's).

b) 25-Amino-3-triisopropylsilyloxy-24-homo-19-nor-cholest-1,3,5(10),16-tetraene [Formula (I) - R = H, R^1 = R^2 = CH_3, R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y = $(CH_2)_4$, Δ16 double bond

Anhydrous cerous chloride was prepared by heating $CeCl_3.7H_2O$ (2 g) *in vacuo* (<0.1 mm Hg) first at 70° for 1 hour, then at 110° for 1 hour and finally at 145° for 2½ hours. Thus-obtained anhydrous cerous chloride (256 mg) was heated *in vacuo* at 130° for 2 hours, cooled, then suspended in tetrahydrofuran (3 ml); the resulting mixture was kept overnight with stirring. The suspension was cooled to -78° and then treated with methyl lithium (0.86 ml of a 1.4 M solution in ether). The mixture was stirred for 15 minutes at -78°, 15 minutes at 0°, then cooled to -78° and the nitrile from (a) above (84 mg) in tetrahydrofuran (2 ml + 1 ml wash) was added dropwise. After a further hour at -78° (TLC control), ammonium hydroxide was added and the mixture was warmed to room temperature and filtered through Celite (methylene chloride wash). Removal of the solvents gave the title compound (67 mg, isolated by chromatography): IR ν_{max} 1620 cm^{-1}; NMR ($CDCl_3$) δ 0.96 (s, 18-H's), 0.99 (21-H's), 1.25 (25-H's), 5.2 (bs, 16-H), 6.5 and 6.95 (m, 1-, 2- and 4-H's).

c) 25-Amino-3-triisopropylsilyloxy-24-homo-19-nor-cholest-1,3,5(10),16-tetraene, acetyl amide [Formula (I) - R = CH_3CO, R^1 = R^2 = CH_3, R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y = -$(CH_2)_4$, Δ16 double bond]

The amine from (b) above (67 mg) in dichloromethane (2 ml) containing pyridine (0.475 ml) and acetic anhydride (0.475 ml) was stirred for 4 hours, whereafter the mixture was diluted with dichloromethane, treated with aqueous sodium bicarbonate, and stirred overnight. Work up afforded the title compound (70 mg, isolated by

preparative TLC): IR ν_{max} 1690, 1620, 1600-1450 cm^{-1}; NMR ($CDCl_3$) δ 0.96 (s, 18-H's), 0.99 (21-H's), 1.25 (25-H's), 1.9 (s, COCH's) 5.0 (s, NH), 5.15 (bs, 16-H), 6.5 and 6.95 (m, 1-, 2- and 4-H's).

d) 25-Amino-3-hydroxy-24-homo-19-nor-cholest-1,3,5(10),16-tetraene, acetyl amide [Formula (I) - R = CH_3CO, R^1 = R^2 = CH_3, R^3 = α-CH_3, R^4 = H, Y = $(CH_2)_4$, Δ16 double bond]

The silyl compound from (c) above (70 mg) in tetrahydrofuran (1.5 ml) was desilylated by treatment overnight with tetrabutylammonium fluoride (1.3 ml of a 1 M solution in tetrahydrofuran). The crude product (40 mg) was purified by TLC to give the title compound (27 mg): NMR ($CDCl_3$) δ 0.76 (s, 18-H's), 0.95, 1.0 (21-H's), 1.3 (25-H's), 1.9 (s, COCH's), 5.1-5.3 (m, NH, 16-H), 6.5 and 6.95 (m, 1-, 2- and 4-H's).

Example 2

a) 3-Triisopropylsilyloxy-23,23a-bis-homo-19-nor-chol-1,3,5(10)-triene-24-nitrile [Formula (II) - R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y = $(CH_2)_4$]

The bromide from Preparation 2(c) (130 mg) was treated with α-lithio-acetonitrile as in Example 1 (a) to give the title compound (140 mg crude, 65 mg after chromatography): IR ν_{max} 2250 cm^{-1}; NMR ($CDCl_3$) δ 0.80 (s, 18-H's), 6.5 and 6.95 (m, 1-, 2- and 4-H's).

b) 25-Amino-3-triisopropylsilyloxy-24-homo-19-nor-cholest-1,3,5(10)-triene [Formula (I) - R = H, R^1 = R^2 = CH_3, R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y = $(CH_2)_4$]

The nitrile from (a) above (65 mg) was treated with methyl cerous chloride as in Example 1 (b) to give the

title compound (58 mg): NMR ($CDCl_3$) δ 0.80 (s, 18-H's), 1.3 (s, 25-H's), 6.5 and 6.95 (m, 1-, 2- and 4-H's).

c) 25-Amino-3-triisopropylsilyloxy-24-homo-19-nor-cholest-1,3,5(10)-triene, acetyl amide [Formula (I) - R = CH_3CO, R^1 = R^2 = CH_3, R^3 = α-CH_3, R^4 = $(i\text{-}Pr)_3Si$, Y = $(CH_2)_4$]

The amine from (b) above (58 mg) was acetylated as in Example 1 (c) to give the title compound (57 mg): NMR ($CDCl_3$) δ 0.80 (s, 18-H's), 1.3 (s, 25-H's), 1.9 (s, COCH's), 5.1 (s, NH), 6.5 and 6.95 (m, 1-, 2- and 4-H's).

d) 25-Amino-3-hydroxy-24-homo-19-nor-cholest-1,3,5(10)-triene, acetyl amide [Formula (I) - R = CH_3CO, R^1 = R^2 = CH_3, R^3 = α-CH_3, R^4 = H, Y = $(CH_2)_4$]

The silyl ether from (c) above (57 mg) was desilylated as in Example 1 (d) to give the title compound (51 mg crude, 15 mg purified by TLC): NMR ($CDCl_3$) δ 0.80 (s, 18-H's), 1.3 (s, 25-H's), 1.9 (s, COCH's), 5.0-5.15 (s, NH), 6.5 and 6.95 (m, 1-, 2- and 4-H's).